REMARKS

Claims 29, 51 and 52 are pending. No claims are allowed. Claims 1-28 and 30-50 were previously cancelled.

Applicants have eliminated "(+/-4)" from Claim 29 so that only the 16 and 30 kDa proteins are claimed. This will eliminate the issues in relation to the written description and enablement rejections under 35 USC 112, first paragraph.

Liang et al. (1998) provides no motivation to use the 30 kDa protein of *Sarcocystis neurona* in a method of producing passive immunity against *Sarcocystis neurona*. Since many horses exposed to *Sarcocystis neurona* do not have clinical signs of EPM but have immunity to *Sarcocystis neurona*, the serum antibodies are likely effective for protecting against the parasite. It would appear reasonable to believe that horses with EPM have inadequate response to the parasite which is not sufficient serum antibodies to prevent entry of the parasite into the CFS and that boosting the antibodies against the 16 and 30 kDa antigens would provide a sufficient boost to an infected horses' immune response to inhibit entry of the parasite into the CSF.

Attached is a Declaration dated April 1, 2003, filed with Application Serial No. 09/670,096 (My Ref: MSU 4.1-526), now on Appeal, which demonstrates that both the 16 and 30 kDa antibodies together were more neutralizing than either antibody alone. Thus, the claimed invention is unobvious to one skilled in the art for this reason. Reconsideration is requested.

It is now believed that Claims 29, 51 and 52 are in condition for allowance. Entry of this Amendment is requested as placing the claims in condition for allowance or in better condition for Appeal. Applicants are merely reducing the issues if an Appeal is necessary.

Respectfully,

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Enclosure: Declaration Under 37 CFR 1.132
dated April 1, 2003

APPENDIX B

MSU 4.1-526
Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No. 09/670,096 Group Art Unit: 1645

Filing Date: 2000 September 26

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Padmavathi Basker, Ph.D.

BOX AF

Commissioner of Patents and Trademarks

Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear sir:

Alice J. Murphy states as follows.

(1) That she is an inventor of the invention in the above entitled application.

(2) That she performed an experiment in East Lansing, Michigan at Michigan State University (assignee of the present invention) to determine the neutralizing ability of antibodies against the 16 and 30 kDa antigens. The results showed that cerebral spinal fluid (CSF) from horses infected with *Sarcocystis neurona* which contained only antibodies that were strongly reacting against the 30 kDa antigen was neutralizing as was CSF which contained only antibodies that were strongly reacting against the 16 kDa antigen.

(3) That the experiment used CSF samples isolated from three horses infected with *Sarcocystis neurona*. CSF from the first infected horse contained antibodies which strongly

reacted against both the 16 kDa and 30 kDa antigens, CSF from the second infected horse contained antibodies which strongly reacted against only the 30 kDa antigen, and CFS from the third infected horse contained antibodies which strongly reacted against only the 16 kDa antigen. The controls for the experiment consisted of CSF from a horse from India known not to be infected with *Sarcocystis neurona* and Tris-buffered saline (TBS) containing 5% fetal bovine serum (FBS). The first horse was also culture positive for *Sarcocystis neurona*. Neural tissue from the horse at necropsy was ground up and inoculated into the media on equine dermal cells in culture. The media was replaced after 24, 48, and possibly 72 hours post inoculation and then weekly thereafter. The first plaque was seen on day 29 after inoculation. The merozoites from the plaques were subsequently identified as *Sarcocystis neurona* by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Horses 2 and 3 had clinical signs which suggested the horses were infected with *Sarcocystis neurona*.

(4) That the experiment was performed as follows.

(a) Merozoites of *Sarcocystis neurona* from culture were washed in Tris-buffered saline (TBS) twice to remove media. The merozoites had been previously obtained from neural tissue from a Michigan horse infected with *Sarcocystis neurona*. The identity of the merozoites had been confirmed by PCR and RFLP.

(b) The washed merozoites were diluted in TBS and 35 μL was added to each of the 6 tubes comprising each of the horse groups. To test viability of the merozoites, 35 μL of the merozoites were affixed to a slide by cytospin (two replicates) and stained. There appeared to be about 20 to 30 viable merozoites per 35 μL. The stained cytospin provided an idea of the number of normal appearing and potentially viable merozoites per 35 μL aliquot. To confirm the viability of the merozoites, a real viability test was performed as follows. 70 μL of the merozoites were added directly to a 25 mL flask of confluent equine dermal cells. An additional 70 μL of merozoites were washed and spun twice in the same manner as the test samples. The pellet was

suspended in media and divided between two 25 mL flasks of confluent equine dermal cells. Two hours after inoculating the flasks, moving (spinning as they do when they are "drilling" into a cell) merozoites were seen in all three flasks. In addition, all three flasks developed plaques. Plaques consisted of a minimum of three rounded-up cells contiguous with one another to a maximum of a bare area of surface surrounded by rounded-up cells. (Cells round up when infected and come loose off the well or flask when the cell is heavily laden with parasite or the cell bursts from the parasite load. Since infective merozoites tend to move only into neighboring cells (unless one shakes up the flask which happens when the media is changed), bare areas surrounded by rounded up cells in older plaques are seen). The test for real viability confirmed that the merozoites used in the experiment described herein were viable.

(c) The CSF sample from horse 1 was diluted 1:10, 1:20, 1:40, 1:80, 1:160 with TBS and the CFS samples from horses 2 and 3 were diluted 1:10, 1:20, 1:40, 1:80 with TBS. 200 µL of undiluted CSF and each dilution of CSF was each added to a tube of merozoites. The controls consisted of undiluted Indian horse CSF and TBS containing 5% FBS. There were six replicates of each of the samples and controls.

(d) All the samples and controls were incubated for one hour at 37° C.

(e) Each tube was centrifuged for 4 minutes at 1000 xg to pellet the merozoites. The supernatant fraction was removed and the merozoites were washed by resuspending the merozoite pellets in 300 µL TBS and centrifuging to pellet the merozoites and removing the supernatant fraction. Two washes were performed.

(f) After the final wash, the merozoite pellets were each resuspended in 200 µL of media and then each suspension was added to a well of a six–well plate of a monolayer of equine dermal cells which was just confluent.

(g) The plates were gently swirled to distribute the merozoites over the

monolayer and the cells then incubated at 37° C in a 5% CO_2 atmosphere. The media was replaced after 24 hours and then replaced weekly thereafter.

(h) Any plaques which formed were counted at five and six weeks post inoculation.

(5) That the results of the experiment are shown in Table 1; that the results show that the CSF containing antibodies against either antigen was separately neutralizing when used undiluted compared to the controls; that the results further show that CSF containing both antibodies was neutralizing even when used at a 1:10 dilution; and, that the results show that the neutralizing ability of the undiluted CSF from all three infected horses appears to be significant as was the 1:10 dilution of the CSF from the first horse.

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Table 1

Sample	Dilution	Mean No. Plaques (SE) at 5 weeks post inoculation	Mean No. Plaques (SE) at 6 weeks post inoculation
1 (Anti-16 & -30)	undiluted	4.3 (0.5)	20.0 (1.2)
	1:10	3.2 (0.6)	30.0 (1.8)
	1:20	6.0 (0.6)	55.5 (2.7)
	1:40	7.2 (0.4)	54.2 (2.8)
	1:80	9.0 (0.6)	52.7 (1.7)
	1:160	8.8 (0.4)	54.5 (2.3)
2 (Anti-30)	undiluted	3.7 (0.4)	37.5 (2.4)
	1:10	7.8 (0.3)	57.5 (2.5)
	1:20	6.8 (0.3)	53.2 (3.0)
	1:40	8.3 (0.8)	53.2 (1.7)
	1:80	9.8 (0.4)	57.8 (3.1)
3 (Anti-16)	undiluted	4.0 (0.6)	36.3 (2.1)
	1:10	7.7 (0.8)	55.3 (2.5)
	1:20	8.7 (0.6)	58.3 (3.1)
	1:40	8.7 (0.9)	55.5 (2.6)
	1:80	7.7 (0.8)	49.2 (2.4)
Indian horse	undiluted	8.7 (0.4)	56.8 (2.7)
5% FBS	undiluted	8.5 (0.6)	54.8 (3.2)

(6) That the results of Liang *et al.*, published in Infection and Immunity 66: 1834-1838 (1998), which shows that antibodies against the 30 kDa antigen in serum or CSF from horses infected with *Sarcocystis neurona* are not neutralizing, are not consistent with the results described herein and are not believed to be correct.

(7) That the undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Alice J. Murphy

Alice J. Murphy

Date: 4-17-03